Arsenic Reduction in Drinking Water and Improvement in Skin Lesions: A Follow-Up Study in Bangladesh

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Abbreviations: As, Arsenic; BMI, Body Mass Index; EPA, Environmental Protection Agency; GEE, Generalized Estimating Equations; ICP-MS, Inductively Coupled Plasma Mass Spectroscopy; LOD, Limit of Detection; NIST, National Institute of Standards and Technology; WHO, World Health Organization

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Abstract

Background: Chronic exposure to arsenic is associated with skin lesions. However, it is not known whether reducing arsenic exposure will improve skin lesions.

Objectives: To evaluate the association between reduced arsenic exposures and skin lesion recovery over time.

Methods: A follow-up study of 550 individuals was conducted in 2009-2011 on a baseline population of skin lesion cases (N=900) previously enrolled in Bangladesh in 2001-2003. Arsenic in drinking water and toenails, and skin lesion status and severity were ascertained at baseline and follow-up. Logistic regression and generalized estimating equation (GEE) models were used to evaluate the association between log10-transformed arsenic exposure and skin lesion persistence and severity.

Results: Water arsenic concentrations decreased in this population by 41% overall, and 65 individuals who had skin lesions at baseline had no identifiable lesions at follow-up. Every log10 decrease in water and toenail arsenic was associated with 22% (odds ratio (OR) = 1.22; 95% CI: 0.85, 1.78) and 4.5 times (OR = 4.49; 95% CI: 1.94, 11.1) relative increase in skin lesion recovery in adjusted models, respectively. Additionally, lower baseline arsenic levels were significantly associated with increased odds of recovery. A log10 decrease in toenail arsenic from baseline to follow-up was also significantly associated with reduced skin lesion severity in cases over time (mean score change = -5.22 units; 95% CI: -8.61, -1.82).

Conclusions: Reducing arsenic exposure increased the odds that individual with skin lesions would recover or show less severe lesions within ten years. Reducing arsenic exposure must remain a public health priority in Bangladesh and in other regions affected by arsenic contaminated water.

Introduction

Globally, millions of people are exposed to arsenic from drinking contaminated water (Kinniburgh et al. 2001). Inorganic arsenic is classified as a Group 1 human carcinogen (Humans 2004) and chronic exposure to arsenic in drinking water is associated with increased risk of skin, bladder, lung and kidney cancer (Fernandez et al. 2012; Guo et al. 2001; Hopenhayn-Rich et al. 1998; Wu et al. 1989). Chronic exposure to arsenic is also associated with increased risk of skin lesions, cardiovascular diseases, lung function, hypertension, reproductive and neurological disorders (Chattopadhyay et al. 2010; Chen et al. 2009; Milton et al. 2005; Rahman 2002).

Arsenic-contaminated groundwater in Bangladesh is a public health concern due to the use of shallow tubewells as part of a public health campaign to reduce the burden of water-borne diseases (Bagla and Kaiser 1996; Chakraborty and Saha 1987). It is estimated that 46% and 27% of the population in Bangladesh was exposed to arsenic concentrations greater than the WHO recommended limit of 10μg/L and greater than the Bangladesh standard of 50μg/L, respectively (Kinniburgh and Smedley 2001). Efforts to remediate arsenic contaminated water in Bangladesh are ongoing and include testing tube wells, labeling unsafe wells, installing new arsenic-free water sources, and point-of-use filters (Joya et al. 2006; UNICEF 2008).

Skin lesions are the first visible symptom of chronic arsenic exposure (Chakraborty and Saha 1987; Rahman et al. 2001; Tseng et al. 1968) and considered to be precursors of arsenic-induced cancers because they are highly associated with skin cancers and other malignancies, and are highly associated with arsenical skin cancers (Cuzick et al. 1982; Cuzick et al. 1984; Cuzick et al. 1992; Tseng 1977). There is good evidence from prospective cohort studies conducted in Bangladesh that the incidence of skin lesions increases with arsenic levels over time (Argos et al. 2011), but few studies have examined whether skin lesions improve if arsenic exposures are

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reduced. Additionally, most previous studies have evaluated the association between arsenic exposure and skin lesions as a dichotomous outcome only, without also considering the severity of skin lesions.

In 2009 we followed up individuals who participated in a case-control study (2001-2003) of environmental and genetic risk factors for arsenic-related skin lesions. Our main objective was to determine if arsenic exposures decreased over time, and if arsenic reduction was associated with a reduction in skin lesions. We estimated associations between changes in arsenic exposure and the prevalence and severity of skin lesions at follow-up among participants who had skin lesions at baseline.

Methods

Study population

In 2001-2003 (baseline), we enrolled 900 individuals who were diagnosed with arsenic-related skin lesions and 900 age- and sex-matched controls in Pabna, Bangladesh into a case-control study to identify factors that influence susceptibility to arsenic-related skin lesions, as previously described by Breton et al (Breton et al. 2006). In 2009-2011, a follow-up study successfully re-contacted 845 (93.9%) of the original 900 participants with skin lesions, of whom 550 (61.1%) cases agreed to participate in the follow-up study. The main reasons for non-participation were refusal (53%), moved away from the district (38%), and mortality (9%).

All individuals in the baseline case-control study participated in Dhaka Community Hospital's arsenic awareness program which provided information on the health effects of arsenic exposure, actions that individuals could take to reduce their exposure, and the importance of a diet rich in fruits and vegetables. Dhaka Community Hospital and their affiliated clinics also worked with

impacted villages to install arsenic-free water sources through shallow dug wells, larger Indira wells, filtered surface water and rainwater harvesting (Joya et al. 2006; Quamruzzaman et al. 2001). Efforts to provide new water sources were targeted for households that were considered highly-exposed (>50 μg/L).

At the time of follow-up, all participants underwent a physical examination and skin lesion status was reassessed by the same physician who conducted the baseline exam in 2001–2003 and who was blinded to the arsenic concentration in the participants' drinking water. The follow-up study protocol was approved by the Institutional Review Boards at the Harvard School of Public Health and Dhaka Community Hospital. Informed consent was provided by every participant prior to participation in the follow-up study.

Questionnaires and interviews

Trained interviewers administered questionnaires to collect socio-demographic information, drinking water history, medical history, lifestyle factors, dietary information, water consumption (liters of water/liquid ingested per day), and residential history including identification of the primary water source (tube well), years of use, and use of a previous tube well. Interviewers in the follow-up study were blinded to the participants' disease status and arsenic exposure at baseline.

Exposure assessment

We collected a 45ml water sample from each participant's primary drinking source in a 50ml falcon tube and added one drop (0.1 ml) of pure trace metal grade nitric acid to preserve the samples for trace metal analysis. Samples were stored at room temperature prior to analysis. Analysis of each sample for arsenic concentration was completed using Environmental

Protection Agency (EPA) method 200.8 with Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) by the same laboratory that performed the baseline measurements (Environmental Laboratory Services, North Syracuse, New York). For quality control, instrument performance was validated using repeated measurements of standard reference water (PlasmaCAL multi-element QC standard #1 solution, SCP Science, Canada) with an average percent recovery of 95%. Ten percent of the samples were randomly selected and analyzed in duplicate to confirm reliability. The average percent difference between duplicates was 2.5%. The limit of detection (LOD) was 1µg As/L. Samples with below the LOD were assigned a value of 0.5µg As/L.

Nail clippings were collected from each participant, placed in an envelope, and stored at room temperature in a dry location. External contamination was removed by sonicating the nails with 1% Triton X-100 solution for 20 minutes (Sigma-Aldrich, Inc.). Samples were digested at room temperature for at least 36 hours following the same protocols as the baseline case-control study (Amarasiriwardena et al. 1998). Total arsenic was measured using inductively coupled plasma mass spectrometry (ICP-MS Model 6100 DRC, PerkinElmer, Norwalk, CT) and each sample was subjected to five replicate analyses. The average detection limit was 0.02µg/g. None of the samples were below LOD. Instrument performance and the digestion process were validated using standard reference material water (NIST 1643d and NIST 1643e Trace Elements in Water; National Institute of Standards and Technology, Gaithersburg, MD). The average percent recovery of NIST was 86.5%, and average percent hair SRM across batches was 73.6%.

Outcome assessment

Two different metrics for skin lesions were assessed: as a dichotomous skin lesion status (yes/no) and continuous skin lesion severity score.

Dichotomous physician-diagnosed skin lesions

Melanosis (yes/no) was defined as any diffuse or spotted lesion characterized by dark pigmentation on the face, oral cavity, neck, upper and lower limbs, chest or back. Keratosis (yes/no) was defined as any diffuse or spotted lesion characterized by hard and roughened skin elevations observed on the palm or dorsum of the hands and/or the sole or plantar of the foot. Hyperkeratosis (yes/no) was defined as extensively thickened keratosis observed on the palm or dorsum of the hands and/or the sole or plantar of the foot that are easily visible from a distance. Leukomelanosis (yes/no) was defined as depigmentation characterized by black and white spots present anywhere on the body. At follow-up, Persistent Cases were defined as participants who had at least one type of arsenic-induced skin lesion (melanosis, keratosis, hyperkeratosis or leukomelanosis) at baseline and at follow-up. At the time of the follow up physical examination, 65 (11.8%) individuals who had skin lesions at baseline had no visible skin lesions and were classified as 'Recovered Cases'.

Continuous skin lesion severity score

To assess the severity of skin lesions, the study physician noted the presence of any skin lesion and assigned a score of 0, 1 or 2 (indicating no lesion, a mild lesion, or a severe lesion, respectively) in each one of 11 specified anatomical regions for diffuse and spotted melanosis (face, oral cavity, neck, arm, dorsum, palm, chest, back, leg, plantar and sole; maximum possible score of 22+22=44), each one of 4 specified anatomical regions for diffuse and spotted keratosis (palm, dorsum, sole and plantar; maximum possible score of 8+8=16) and hyperkeratosis (palm, dorsum, sole and plantar; maximum possible score of 8), , and also noted the presence/absence of leukomelanosis anywhere on the body (maximum score of 1), and then summed the individual scores across the anatomical regions to create a continuous severity score that ranged from 0 to

69. This overall severity score takes into account the possibility for an individual to have more than one type of skin lesions, each of different severity. This approach is adapted from a method used by Ahsan et al. (2006) to quantify arsenic-induced skin lesions (Ahsan et al. 2006) that was based on methodology originally used to determine the extent of body involvement in burn patients. Color photographs of representative mild (severity score = 1) and severe (severity score = 2) skin lesions are shown in Supplemental Material, Figure S1. Photographs of lesions were taken for all the skin lesion cases at baseline and a random sample (5%) of Persistent Cases were independently scored by a dermatologist to evaluate inter-rater reliability, which indicated mean consistency of 72% for the overall scores for each participant in the subsample with previous diagnosis.

Statistical analysis

Our analysis was limited to individuals with skin lesions diagnosed at baseline in 2001-2003 who had follow-up information in 2009-2011. All covariates were determined at both baseline and follow-up. Covariates such as age, gender, smoking status and betel nut consumption were compared between followed-up and non-participating cases, as well as Persistent Cases and Recovered Cases at follow-up, using Fisher's exact test for categorical variables, Welch's t-test for normally-distributed variables, and Wilcoxon rank sum test with continuity correction for non-normally distributed continuous variables. All arsenic variables were log10-transformed due to arsenic being right-skewed. Arsenic change for participant i ($\Delta As_i = \log10As_{i0} - \log10As_{i1}$) was defined as the reduction of arsenic levels between follow-up (As_{i1}) and baseline arsenic levels (As_{i0}).

First, we evaluated the association between reduction in arsenic levels and skin lesion recovery (Recovered Cases, N=65 versus Persistent Cases, N=485) using logistic regression models:

$$\text{logit}(\pi_i)= \beta_0+ \beta_1 \log10As_{i0} + \beta_2 \Delta As_i + \alpha^T Z_i, \quad [1]$$

where π_i is the probability of full recovery status of subject i at follow-up; β_0 is the intercept; β_1 is the log odds of skin lesion recovery associated with a log10-unit increase in As_{i0}; β_2 is the log odds of skin lesion recovery associated with a log10-unit decrease in ΔAs_i; α^T is a row vector of regression coefficient estimates for covariates at follow-up (T denotes vector transpose) and Z is a vector of covariates at follow-up. Covariates included in the final model were selected a priori, including age (continuous), gender, education ($\leq$ primary, secondary–college, $\geq$ graduate), body mass index (continuous), smoking status (never versus ever or current smoker), chewing betel nuts (yes or no) at follow-up.

Second, we used linear regression with generalized estimating equations (GEE) to estimate the association between changes in skin lesion severity between baseline and follow-up among all cases in relation to changes in biomarkers of arsenic exposure (water, toenails) over time, controlling for baseline arsenic levels (Diggle et al. 2002). The GEE analysis accounts for within-subject correlation between baseline and follow-up severity scores. It also uses all the observed data of 900 subjects, including those subjects who have both baseline and follow-up data (N=550) and those who only had the baseline data (N=350) to improve analysis power. The model used in the GEE analysis can be written as

$$E(Score_{it}) = \beta_0 + \beta_1 \log10As_{i0} + \beta_2 t_i + \beta_3 \Delta As_{it} + \beta_4 \log10As_{i0}*t_i + \alpha^T Z_i, \quad [2]$$

where $Score_{it}$ is severity score of subject i at time t (0= baseline, 1= follow-up); ΔAs_{it} change in arsenic levels in subject i at time t with $\Delta As_{i0}=0$; and other variables are defined as above. At baseline (t = 0) the model simplifies to $E(Score_{i0}) = \beta_0 + \beta_1 \log10As_{i0} + \alpha^T Z_i$ and at follow-up (t =

1) the model simplifies to $E(Score_{i1}) = \beta_0 + \beta_1 \log10As_{i0} + \beta_2 + \beta_3 \Delta As_{i1} + \beta_4 \log10As_{i0} + \alpha^T Z_i$ such that the change in the severity score between baseline and follow-up for individual i ($\Delta Score_i$) is represented by $E(Score_{i1}) - E(Score_{i0})$, i.e.,

$$E(\Delta Score_i) = \beta_2 + \beta_3 \Delta As_i + \beta_4 \log10As_{i0}. \quad [3]$$

Hence β_1 is the change in mean baseline severity score for every log10-unit increase in baseline arsenic levels; β_3 is the change in ΔScore for every log10-unit decrease in ΔAs_i (i.e., the estimated effect of the change in arsenic levels over time on the change in the severity score over time); and β_4 is the change in ΔScore for every log10-unit increase in baseline arsenic levels (i.e., the estimated effect of baseline arsenic levels on the change in the severity score over time). Table 1 shows that the subjects who dropped out from the study were younger, had a lower BMI and higher arsenic level at baseline. In order to account for potential bias due to dropout of these cases at follow-up, we performed an inverse probability weighted (IPW) GEE model by assigning weights according to each individual's estimated probability of participating in the follow-up study using logistic regression on age, BMI and arsenic exposures at baseline and found virtually unchanged results as the unweighted GEE model. All statistical analyses were conducted using R version 2.13.1 and SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). All tests are conducted as 2-sided and are considered significant with a p-value of less than 0.05.

Results

In total, 61.1% of the 900 cases (n=550) from the original case-control study participated in follow up. At baseline, cases who participated in the follow-up study had higher BMI, used

tubewells that had lower arsenic concentrations, and had lower toenail arsenic than cases who did not participate in follow up ($p < 0.05$) (Table 1).

Sixty-five participants had no skin lesions identified at follow-up (Recovered Cases), whereas 485 participants still had skin lesions (Persistent Cases) (Table 2). At follow up, the Recovered Cases were younger (mean 39.2 years compared with 41.8 years, $p = 0.02$), had higher average BMI (21.0 compared with 20.0, $p = 0.01$) and did not chew betel nuts as frequently (18.5% versus 32.6%, $p = 0.02$) than Persistent Cases. Overall, drinking water arsenic concentrations declined significantly in the follow-up study population (125 ± 227 µg/L at follow-up versus 213 ± 302 µg/L at baseline, p <0.001) (Table 1 and Figure 1). Amongst the Recovered Cases, drinking water arsenic concentrations declined, on average, by 70.5%, from a mean of 105.4 µg/L in 2001 to 31.1 µg/L in 2009, whereas in Persistent Cases there was a mean reduction of only 43.4% from 221.6 µg/L in 2001 to 125.4 µg/L in 2009, even though the absolute change in water arsenic concentrations was greater for the persistent cases than recovered cases (Table 2). Participants exposed at baseline to water arsenic > 50 µg/L (the Bangladesh recommended maximum contaminant level of arsenic) were targeted for mitigation activities, and 40.4% had water arsenic levels < 50 µg/L at follow-up (Table 2). A significantly lower proportion of Recovered Cases were exposed to ≥ 50 µg/L water arsenic at follow-up as compared to Persistent Cases (13.8% versus 32.8%). Toenail arsenic levels were significantly reduced from baseline to follow-up in the Recovered Cases (5.31 µg/g versus 1.95 µg/g) but not in the Persistent Cases (6.29 µg/g vs. 6.07 µg/g) which supports our hypothesis that reduction in arsenic exposure is the main driving force for their recovery. Among Persistent Cases, water arsenic levels also decreased over time by more than 200% (mean decrease of 97µg/L) yet mean

skin lesion severity increased in this group from 16 units in 2001 to 39 units in 2009 (p < 0.05) (Table 2). This increase in skin lesion severity may be due to continued arsenic exposure since the average drinking water arsenic in this group remained at 125 µg/L, and as noted above, there was little change in average toenail arsenic concentrations in Persistent Cases.

The association between reduction in arsenic level and skin lesion recovery (yes/no) in cases at follow-up was assessed using logistic regression (Table 3). For every log10 unit decrease in water arsenic, there was a 1.22 times increase in odds of skin lesion recovery (odds ratio = 1.22; 95% CI: 0.85, 1.78), and for every log10 unit decrease in toenail arsenic, there was a significant 4.49 times increase in odds of skin lesion recovery (odds ratio = 4.49; 95% CI: 1.94, 11.1), after adjusting for age, gender, education, smoking status, betel nuts, BMI and baseline arsenic. In other words, the higher the degree of arsenic levels reduction in both water and toenail, the higher the probability of recovering from skin lesions over time. Significant associations were also seen with baseline arsenic levels. For every log10 unit increase in baseline water arsenic, there was a 41% relative decrease in odds of skin lesion recovery (odds ratio = 0.59; 95% CI: 0.41, 0.81). For every log10 unit increase in baseline toenail arsenic, there was an 80% relative decrease in odds of skin lesion recovery (odds ratio = 0.20; 95% CI: 0.08, 0.44), after adjusting for all other covariates.

Linear regression using generalized estimating equation (GEE) was used to assess the association between longitudinal arsenic level profiles and skin lesion severity profiles while accounting for possible correlation among repeated measures over time within the same subject. For every log10 unit reduction in water arsenic, mean skin lesion severity score was reduced by 0.70 units

(95% CI: -2.18, 0.78), and for every log10 unit reduction in toenail arsenic, mean skin lesion severity score was significantly reduced by 5.22 units (95% CI: -8.61, -1.82), after adjusting for age, gender, education, smoking status, betel nuts, BMI and baseline arsenic (Table 4). For every log10 unit increase in baseline water arsenic, mean skin lesion severity score was reduced by 0.87 units (95% CI: -2.85, 0.18) after adjusting for all other covariates.

Discussion

Our results show that arsenic remediation activities and safe water programs have successfully reduced arsenic exposures in this population. Those individuals who were drinking from a tubewell that exceeded the Bangladesh drinking water standard of >50 µg/L had the most dramatic decreases in arsenic exposure, indicating that these individuals were able to reduce their exposures and that Dhaka Community Hospital's arsenic remediation program was effective at helping to encourage and motivate individuals to change their drinking water sources and behaviors that lead to arsenic exposure. We observed a decrease in drinking water arsenic over time that was associated with increased probability that skin lesions would improve within a period of ten years. These findings highlight the importance and effectiveness of long-term community-based arsenic remediation efforts in Bangladesh.

As expected, we observed that individuals who had lower baseline arsenic exposures had significantly higher odds of recovery at follow-up. Recovered Cases were exposed to lower baseline water arsenic levels than Persistent Cases, and had lower water arsenic levels at follow-up. They also had much greater reduction in toenail arsenic levels compared to Persistent Cases. This suggested that reduction of water arsenic over time, together with lower baseline arsenic

levels, are strongly associated with recovering from skin lesions. We also observed a borderline

significant association between baseline water arsenic and reduced skin lesion severity, which

might be due to the possibility that individuals with higher baseline arsenic were more targeted

to change their water sources and therefore, have reduced skin lesions severity at follow-up.

However, Persistent Cases that were highly exposed at baseline and reduced their exposure to

arsenic contaminated drinking water may need to further reduce their exposures or require a

longer period of time to recovery before full recovery of their skin lesion symptoms are observed.

Very little information is available regarding the effects of reducing arsenic exposure on skin

lesion recovery. An early clinical report that reviewed all prior literature on arsenical cancers

stated that early-stage arsenic-induced lesions can spontaneously disappear when the use of

medications containing arsenic is reduced, but no quantitative population study has yet been

published and the biological mechanisms are still poorly understood (Neubauer 1947). It has

been demonstrated that exposed individuals with hyperkeratosis have significantly higher DNA

damage measured using chromosomal aberration assay in lymphocytes, and less DNA repair

capacity measured using challenge assay in whole blood than those without skin lesions

(Banerjee et al. 2008). Another study also reported that oxidative DNA damage (urinary 8-

OHdG) was repaired and returned to normal in patients who ceased being exposed to arsenic

from accidental oral intake of arsenic trioxide after 180 days (Mahata et al. 2004; Yamauchi et al.

2004). The reduction of water arsenic was significant in our population, especially in the

recovered group. Therefore, repair of DNA damage may explain why we observed reversibility

of skin lesions with lowered arsenic exposures.

In our population, well-switching for individuals exposed to >50 µg/L to a safe well that contains

lower arsenic concentrations was the most common arsenic remediation intervention. Another

prospective study evaluated the effectiveness of this approach by comparing urinary arsenic

concentrations at baseline and two years later, and found a 46% reduction in mean urinary

arsenic concentrations among those who switched wells (Chen et al. 2007). Combined, these

results suggest that well switching is an acceptable remediation strategy that can reduce

individual's exposure to arsenic contaminated drinking water and improve public health

outcomes. Besides remediation activities to reduce arsenic exposure from drinking water,

numerous studies have also shown dietary supplements to play an important role in influencing

arsenic toxicity both in the human population and *in vitro* done in blood cultures (Biswas et al.

2010; Gamble et al. 2006; Gamble et al. 2007; Heck et al. 2007; Tiwari and Rao 2010). However,

a pilot supplementation trial provided randomized subjects with supplements vitamin E,

selenium and the combination for 6 months did not find a significant mean decrease in skin

lesion scores (Verret et al. 2005). Compared to the results from implementing dietary

supplementation, we have shown that lowering the arsenic levels in drinking water may result in

a more significant improvement of skin lesion severity and eventually recovery.

Some limitations of our study include possible exposure misclassification, misclassification of

mild skin lesions, lack of confirmation of recovered cases by an independent physician, and

selection bias due to refused or not followed up study participants. Subjects could have been

drinking from water sources that were unaccounted for since we have only one measurement

water arsenic in any one period, leading to possible exposure misclassification. However, we

also observed associations between reduced toenail arsenic, a biomarker of internal dose, and the

odds of skin lesion recovery. False positive misclassification of mild skin lesions such as melanosis or leukomelanosis was possible and participants who were actually controls were misclassified as cases at baseline only to be re-evaluated as Recovered Cases at follow-up. However, the same physician assessed the cases in both baseline and follow-up. Furthermore, the physician was blinded to participants' baseline case status and arsenic exposures. Lastly, since this was a follow-up of a case-control study with a substantial percentage of participants who were not followed up, there might be potential selection bias if cases that were not followed up had higher arsenic exposures and also developed more severe skin lesions due to arsenic toxicity. However, when we compared baseline characteristics of the two groups (followed-up and non-participating Cases) in Table 1, we found no significant differences in their skin lesion severity scores or risk factors for skin lesions such as smoking and chewing betel nuts.

Our study strengths include a relatively long follow-up period of almost ten years, individual exposure assessment for arsenic which included external environmental exposure from drinking water and cumulative internal dose in toenails as biomarkers at both baseline and follow-up, and skin lesions were assessed on a continuous scale to provide additional information on their severity. Given the follow-up nature of the study design, we were also able to look at changes in arsenic levels on skin lesions. Although there are currently a few prospective studies being performed on the association between arsenic and skin lesions, we are not aware of published studies concerning associations between reductions in arsenic exposures over time and skin lesion recovery or changes in skin lesion severity over time. This is also one of the first studies that evaluated the effects of reducing arsenic exposures via drinking water by assessing skin lesions improvements over time, since arsenic poisoning was first discovered in the 1970s.

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Our results strongly show that reduced arsenic exposures not only increased the odds of skin lesion recovery, but also reduced the severity of skin lesions amongst participants with skin lesions over time. Intervention efforts should be targeted at individuals with skin lesions to reduce their arsenic levels as early as possible to increase their chances of skin lesion recovery. Since arsenic-induced skin lesions are often the first visible symptom of arsenic poisoning and therefore often indicative of more arsenic-related morbidities to follow, the fact that it is reversible by reducing arsenic exposures over a period of time holds promises of full recovery from skin lesions as long as effective remediation efforts are put in place.

Conclusion

In summary, we found a substantial reduction in water arsenic levels in our study population, reflecting considerable efforts to reduce arsenic intake from drinking water in this region over the time period investigated. We found that the reduction in arsenic exposure was associated with increasing odds of recovery from skin lesions, as well as with reduced severity of persistent lesions at follow-up. Future studies with extended follow-up are warranted to assess whether this reduction in the presence and severity of skin lesion results in reduced risks of cancers and other arsenic-related diseases with longer latency periods.

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Table 1. Characteristics of follow up cases (N=550) and non-participating cases (N=350) in Pabna, Bangladesh, 2001-2003.

Characteristic[a]	Followed-up Cases (N=550) Baseline	Follow-up	Non-participating Cases[b] (N=350) Baseline	p-value[c]
Gender				
Males	340 (61.8%)	340 (61.8%)	216 (61.7%)	0.96
Females	210 (38.3%)	210 (38.2%)	134 (38.3%)	
Age, years	34.2 ± 11.8	41.4 ± 12.5	32.5 ± 12.8	0.05
Education				
None-Primary	351 (63.9%)	408 (74.2%)	216 (61.7%)	0.15
Secondary-College	180 (32.8%)	111 (20.2%)	113 (32.3%)	
Graduate and above	18 (3.3%)	31 (5.6%)	21 (6%)	
BMI, kg/m^2	20.2 ± 3.12	20.6 ± 3.10	19.7 ± 2.93	0.01
Smoking Status (Only in males)				
Ever	103 (30.4%)	168 (49.4%)	60 (27.8%)	0.28
Never	236 (69.6%)	172 (50.6%)	156 (72.2%)	
Betel nuts				
Yes	162 (29.6%)	170 (30.9%)	94 (27.0%)	0.46
No	386 (70.4%)	380 (69.1%)	254 (73.0%)	
Water Arsenic, μg/L	212.9 ± 301.9	125 ± 227	263.2 ± 314.3	0.002
Toenail Arsenic, μg/g	6.18 ± 7.95	6.0 ± 8.15	7.33 ± 8.53	0.009
Severity Score	16.5 ± 14.8	34.4 ± 15.6	16.5 ± 15.4	0.67

[a] Data are shown as mean ± standard deviation (SD) for continuous variables or n (%) for categorical variables.

[b] Reasons for non-participation included: 53% refused to participate in follow-up study, 38% moved away and 9% passed away.

[c] p-values comparing baseline characteristics between follow-up and non-participating subjects obtained from Welch's t-test for normally-distributed and Wilcoxon rank sum test with continuity correction for non-normally distributed continuous variables, and Fisher's exact test for categorical variables.

Table 2. Characteristics of Persistent Cases (N=485) and Recovered Cases (N=65) at follow-up in Pabna, Bangladesh, 2009-2011.

Characteristic[a]	Persistent Cases	Recovered Cases	p-value[b]
Gender			
Males	294 (60.6%)	44 (67.7%)	0.13
Females	191 (39.5%)	21 (32.3%)	
Age, years	41.8 ± 12.5	39.2 ± 12.8	0.02
Education Level			
None-Primary	367 (75.7%)	41 (63.1%)	0.09
Secondary-College	92 (19.0%)	19 (29.2%)	
Graduate and above	26 (5.36%)	5 (7.7%)	
BMI	20.0 ± 3.11	21.0 ± 3.03	0.01
Smoking Status (Only in males)			
Ever	145 (49.3%)	23 (50.0%)	0.98
Never	149 (50.7%)	23 (50.0%)	
Betel nuts			
Yes	158 (32.6%)	12 (18.5%)	0.02
No	327 (67.4%)	53 (81.5%)	
Baseline Water Arsenic, µg/L	222 ± 309	105 ± 196	0.004
Follow-up Water Arsenic, µg/L	125 ± 229	31.1 ± 64.6	0.002
Change in Water Arsenic, µg/L	-97.0 ± 331	-47.4 ± 155	0.51
Baseline Water Arsenic ≥ 50 µg/L			
Yes	216 (44.5%)	21 (32.3%)	0.06
No	269 (55.5%)	44 (67.7%)	
Follow-up Water Arsenic ≥ 50 µg/L			
Yes	159 (32.8%)	9 (13.8%)	0.001
No	326 (67.2%)	56 (86.2%)	
Baseline Toenail Arsenic, µg/g	6.29 ± 7.09	5.31 ± 12.7	0.002
Follow-up Toenail Arsenic, µg/g	6.07 ± 8.23	1.95 ± 2.79	< 0.001
Change in Toenail Arsenic, µg/g	-0.43 ± 7.40	-3.14 ± 12.7	0.13
Baseline Severity Score	16.0 ± 14.6	8.14 ± 11.7	< 0.001
Follow-up Severity Score	39.0 ± 9.75	0	-

[a] Data are shown as mean ± standard deviation (SD) for continuous variables or n (%) for categorical variables.

[b] *p*-values comparing Persistent and Recovered Cases obtained from Welch's t-test for normally-distributed and Wilcoxon rank sum test with continuity correction for non-normally distributed continuous variables, and Fisher's exact test for categorical variables.

Table 3. Change in odds of skin lesion recovery at follow-up examination (N=65) for every log10 unit decrease in arsenic concentration between baseline (2001-2003) and follow-up (2009-2011) among baseline cases (N=541) who had follow-up data.

| | Exposure (log10) | Crude | | Adjusted[b] | |
		Change in Odds (95% CI[a])	p-value	Change in Odds (95% CI[a])	p-value
Decrease[c]	Water Arsenic	1.37 (0.98, 1.96)	0.072	1.22 (0.85 1.78)	0.28
	Toenail Arsenic	5.32 (2.38, 12.6)	< 0.001	4.49 (1.94, 11.1)	< 0.001
Baseline[d]	Water Arsenic	0.56 (0.40, 0.77)	< 0.001	0.59 (0.41, 0.81)	0.002
	Toenail Arsenic	0.18 (0.08, 0.39)	< 0.001	0.20 (0.08, 0.44)	< 0.001

[a] CI denoted confidence interval.

[b] Model was adjusted for age, gender, smoking status, betel nuts, education, BMI and baseline arsenic.

[c] Decrease between baseline and follow-up

[d] For every log10 unit increase in baseline arsenic level

Table 4. Decrease in mean severity score associated with a log10 unit decrease in arsenic concentration in cases (N=550) at the follow-up examination (2009-2011) using linear regression fitted using generalized estimating equation (GEE).

	Exposure (log10)	Crude Mean Score Change (95% CI[a])	p-value	Adjusted[b] Mean Score Change (95% CI[a])	p-value
Decrease[c]	Water Arsenic	-0.84 (-2.34, 0.65)	0.27	-0.70 (-2.18, 0.78)	0.35
	Toenail Arsenic	-5.74 (-9.15, -2.33)	< 0.001	-5.22 (-8.61, -1.82)	0.003
Baseline[d]	Water Arsenic	-1.14 (-2.63, 0.36)	0.13	-1.34 (-2.85, 0.18)	0.08
	Toenail Arsenic	0.24 (-3.04, 3.52)	0.89	-0.092 (-3.41, 3.22)	0.96

[a] CI denoted confidence interval.

[b] Model was adjusted for age, gender, smoking status, betel nuts, education, BMI and baseline arsenic.

[c] Decrease between baseline and follow-up

[d] For every log10 unit increase in baseline arsenic level

Figure Legend

Figure 1. Reduction of mean arsenic concentrations in water and toenail between baseline (2001-2003) and follow-up (2009-2011) in the Recovered Cases (N=65), Persistent Cases (N=485) and subjects exposed to >50 µg/L of baseline water arsenic (N=237). P-values were generated using Welch's t-test, [*]$p<0.05$, [**]$p<0.01$, [***]$p<0.001$. Error bars were obtained by 1.96*(standard error of mean). Significant reduction in water arsenic was seen in all three groups.

Figure 1. Reduction of Mean Arsenic Concentrations in Water and Toenail between Baseline (2001-2003) and Follow-up (2009-2011) in the Recovered Cases (N=65), Persistent Cases (N=485) and Subjects Exposed to >50 µg/L of Baseline Water Arsenic (N=237).